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Many questions needed to advance our understanding of the mechanism of injury from high-level NO_2, remain unanswered to date. This is partly due to the limited interest in the toxicity of high-level exposures, and partly due to the public pressure and interest to study the effects of low-(environmental) levels. However, the effects of exposure to high-level NO_2 are of great interest to the military since high levels of NO_2 may be found in combat situations. It is also important to the civilian sector in occupational settings were accidents may occur as in Silo filler accidents. To fill this gap in knowledge, the Department of Respiratory Research, Division of Medicine at Walter Reed Army Institute of Research took the initiative and convened a panel of experts in a symposium to discuss in depth the effects of exposure to high-level nitrogen dioxide. The symposium goals were to address the issues beginning from the chemistry of NO_2 molecule, to the dosimetery of its uptake (isolated lung), to the biological effects of exposure in vivo in small animals (rats), large animals (sheep), and finally in the most relevant species, humans.

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Toxicity of nitrogen dioxide: an introduction

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Abstract

Many questions, needed to advance our understanding of the mechanism of injury from high-level NO_2, remain unanswered to date. This is partly due to the limited interest in the toxicity of high-level exposures, and partly due to the public pressure and interest to study the effects of low- (environmental) levels. However, the effects of exposure to high-level NO_2 are of great interest to the military since high levels of NO_2 may be found in combat situations. It is also important to the civilian section in occupational settings where accidents may occur as in silo filler accidents. To fill this gap in knowledge, the Department of Respiratory Research, Division of Medicine at Walter Reed Army Institute of Research took the initiative and convened a panel of experts in a symposium to discuss in depth the effects of exposure to high-level nitrogen dioxide. The symposium goals were to address the issues beginning from the chemistry of NO_2 molecule, to the dosimetry of its uptake (isolated lung), to the biological effects of exposure in vivo in small animals (rats), large animals (sheep), and finally in the most relevant species, humans.

Keywords: Nitrogen dioxide; Inhaled oxidants; Military exposure; Civilian exposure; Photochemical oxidants

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1. Toxicity of nitrogen dioxide: an introduction

Nitrogen dioxide (NO_2) along with nitric oxide (NO) and ozone (O_3) constitute the most damaging components of photochemical smog that pollutes the urban atmosphere and are called collectively, photochemical oxidants (National Academy of Sciences, Samet and Utell, 1990). Nitrogen dioxide is a nitrogen centered free radical gas with limited solubility in aqueous solutions. In water, NO_2 is hydrolyzed (reactions 1 and 2) to yield nitrous acid (HNO_2), nitric acid (HNO_3), and nitric oxide (NO).

$$2NO_2 + H_2O \longrightarrow HNO_2 + HNO_3 \quad (1)$$

$$2HNO_2 \rightleftharpoons NO + NO_2 + H_2O \quad (2)$$

1.1. Characterization of the pulmonary injury and repair processes

Inhalation of NO_2 at low concentrations results in pulmonary injury. The severity of such injury is dose-dependent. The injury is characterized morphologically, by loss of ciliated cells in the airways, and degeneration of alveolar epithelial type I cells leaving the basement membrane denuded (Evans et al., 1973; Hayashi et al., 1987; Guidottio, 1980). The disappearance of epithelial type I cells, which normally covers 97% of the alveolar surface, will presumably release epithelial type II cells from contact inhibition which would then undergo cellular hypertrophy and hyperplasia to replace the damaged type I cells. Within 48-72 h, the denuded basement membrane will be repopulated again, but with the rapidly proliferating type II cells (Evans et al., 1973; Hayashi et al., 1987; Yuen and Sherwin, 1971). If the NO_2 stimulus is discontinued, the newly formed cuboidal, metabolically active, and resistant type II cells would undergo a transformation to the large, flat, metabolically less active, and sensitive type I cells. In the airways lost ciliated cells will be replaced with non-ciliated Clara cells, and in the capillaries, pinocytotic vesicles would appear in the endothelium followed by interstitial edema and alveolar edema if the injury is severe enough (Hayashi et al., 1987). Evans (1982) assessed quantitatively the changes in alveolar cell population by comparing the labeling index of type II cells (from the rate of tritiated thymidine incorporation into newly synthesized DNA) to the destruction of type I cells (assessed by measuring the area of the basement membrane not covered with cells) over time. In that study, Evans observed that the changes form a linear relationship (Fig. 1). i.e., the rate of type I cell degeneration is proportional to the rate of type II proliferation. In another experiment of the same report, following the time course of alveolar type II cell proliferation showed that cell division increase after NO_2 exposure relative to air controls reaching a peak after about 48 h, thereafter the rate of

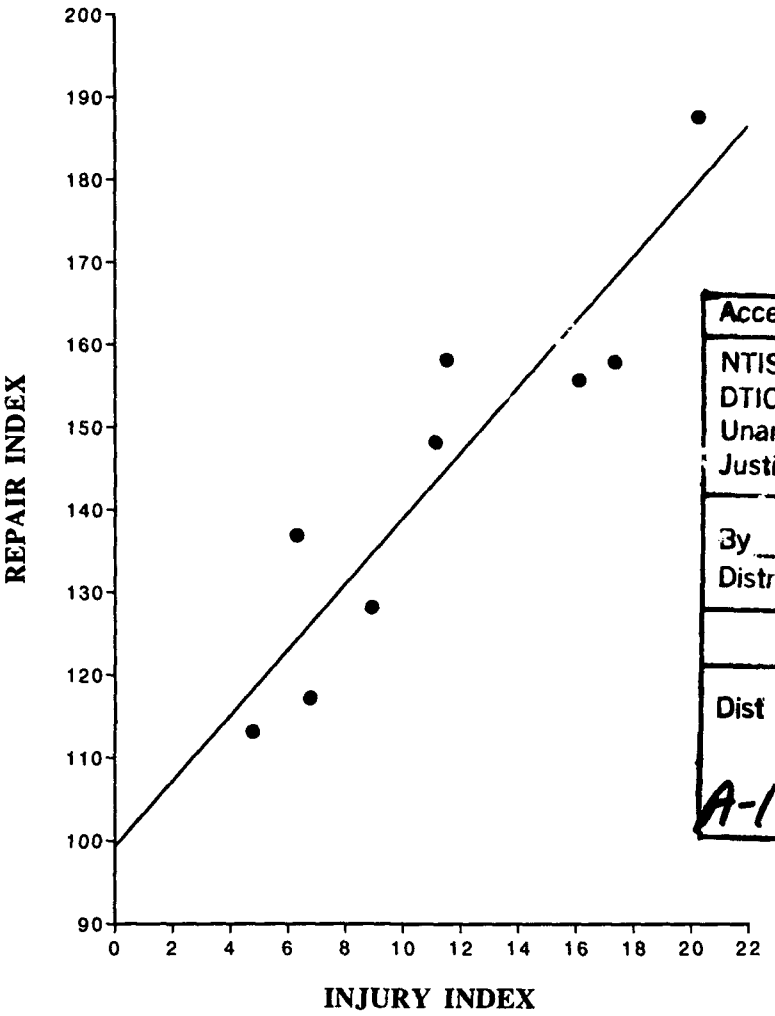

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Fig. 1. Correlation between the Injury Index (defined as the area of assement membrane not covered with type I cells) and the Repair Index (defined as the ratio of labeled type II cells/1000 alveolar cells) after a continuous exposure to 15 ppm NO_2 for 24 h. Modified from Evans (1982).

cell division decrease steadily to control level. The increase in type II proliferation was also found to be dependent on NO_2 exposure level as shown in Fig 2.

Biochemically, Mustafa and Lee (1976) observed that in animals exposed to O_3, the activities of several enzymes tended to increase in lung tissue homogenate after exposure. This pattern was found later by Mustafa as well as by others (Mustafa and Tierney, 1978; Ospital et al., 1981; Sagai et al., 1982; Mustafa et al., 1980), to occur also with NO_2. These activities included

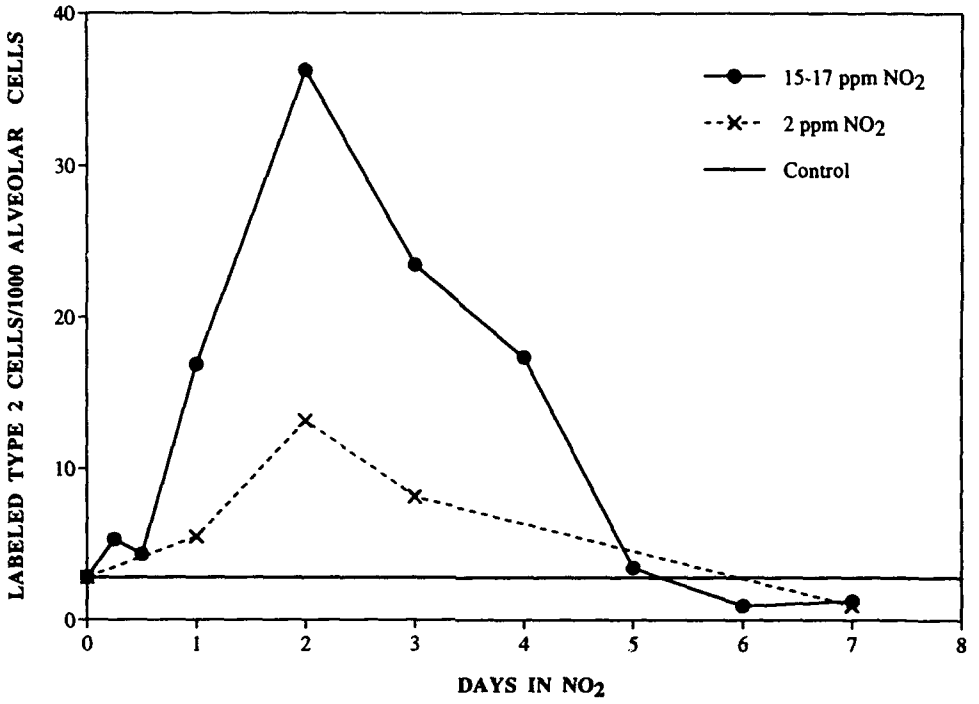

Fig. 2. Proliferation of alveolar type II cells as a function of time and NO_2 exposure level. From Evans (1982) by permission.

those of antioxidant enzymes such as glutathione peroxidase, glutathione reductase, glutathione disulfide transhydrogenase, glucose 6-phosphate dehydrogenase, and NADP-dependant isocitrate dehydrogenase. It included also substrates associated with oxidative stress such as non-protein sulfydryls, as well as oxygen consumption. The observed increases in enzyme activity and substrates were also dose-dependant (Fig. 3).

1.2. General model for the lung response to inhaled oxidants

Based largely on Mustafa's and Evans' observations, a general hypothetical injury model was proposed to describe the pulmonary injury and repair response to low-level oxidant inhalation. The model can describe both the morphological and biochemical responses over time (Elsayed, 1993). In the model, (Fig. 4), during the initial stages of exposure (approximately within the first 24 h), oxidant inhalation causes damage and degeneration of type I cells and decreased metabolic activities below control level reflecting what can be called an 'Injury Phase.' This decline in response is then followed by a gradual increase reaching a peak that plateaus within 48-72 h as type II cells proliferate and cover the basement membrane. A possible inflamma-

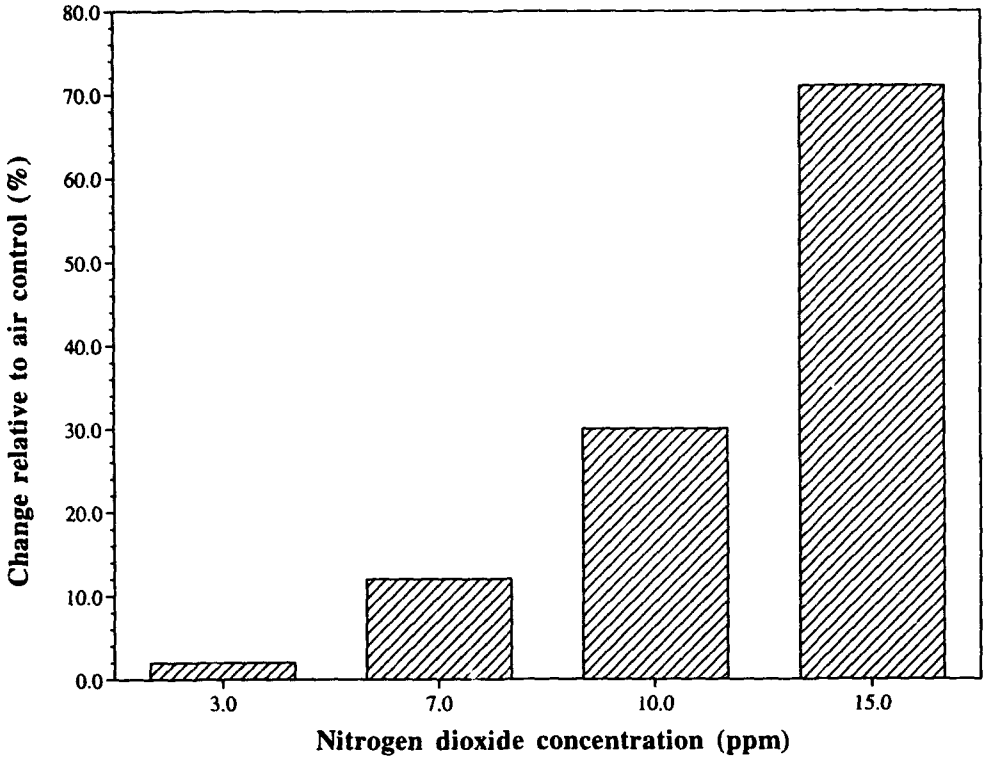

Fig. 3. Dose-dependant changes in the activity of glucose 6-phosphate dehydrogenase in the lungs of rats exposed to NO_2. Modified from Mustafa et al., 1980.

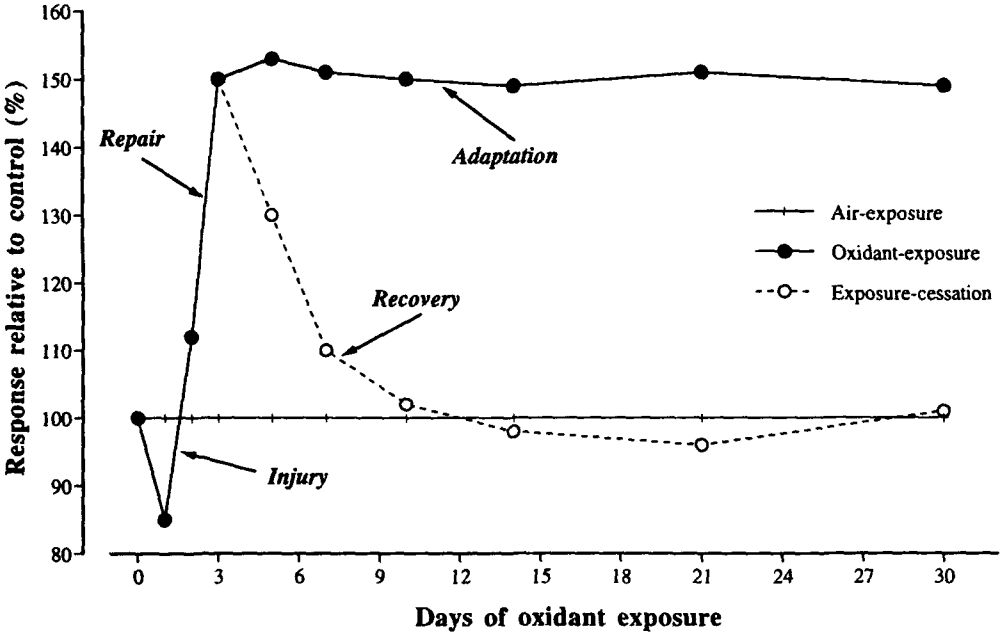

Fig. 4. A hypothetical model of the pulmonary response to inhaled oxidants applicable to both biochemical and morphological changes and is capable of describing the injury and repair processes in the lung. Modified from Elsayed (1993).

tory response associated with this stage, would also contribute to the increased biochemical activities. This increase in response may reflect an adaptive or 'Repair Phase.' The magnitude of increase above control level, in general, would be proportional to the exposure dose. However, this correlation holds up to certain exposure levels, thereafter massive destruction takes place overwhelming the system and resulting in cell death. If however, the exposure is ceased, the response will decline and eventually return to control level.

1.3. Military vs. civilian NO_2 exposures

While the civilian's environment can be protected (or the attempt is made to protect it !) through various laws and regulations, protecting the soldiers environment is a more complicated task and is limited to occupational safety during training and operation of weapon systems. During combat, however, this goal is further reduced by the need to survive and to win battles and wars. Limited as it is, occupational and environmental safety of the soldiers lags behind its civilian counterpart due to the faster pace of developing new weapon systems particularly during the cold war and arms race. As a result, the toxicity of several environments may not have been adequately defined or understood before the soldiers were exposed to them. This may not be due to intentional oversight, but rather the result of many factors such as: (a) lack of knowledge of the toxicity of many agents and the interaction among them when they are present in combination, which is a relevant issue for both the military and civilian sectors; (b) the trade-off that occurs in search of a balance in procuring weapon systems between efficiency to defeat the enemy, and safety of its operators (c) extrapolation of existing data from environmental civilian levels to occupational and military levels before validation under pressure of speed and expediency. The dichotomy between military and civilian environmental standards can be seen from the following example. It has long been recognized that low environmental concentrations of NO_2 (0.5–10 ppm) for relatively longer periods (hours to days) has relatively low toxicity. However, during combat, the soldiers in defeated armored vehicles and structural enclosures are at risk of exposure to very high concentrations of NO_2. For example, in an armored vehicle, levels as high as 1000–2000 ppm were recorded, lasting for a very short period (seconds), but within a few minutes the level was reduced to 100-200 ppm, and within 5–10 minutes, it reached non-toxic levels. Although the effect of acute exposures to such high levels of NO_2 has been examined in animals (Stavert and Lehnert, 1989,1990; Januszkiewicz et al., 1992), the effects on humans, is still fragmentary. Moreover, the long-term effects of exposure to such very high levels of NO_2 albeit for very short periods, have not been addressed yet. There is also a significant lack of knowledge about the correlation between the exposure to high energy impulse noise (blast overpressure)

which is known to cause very serious, auditory and non-auditory injuries, and high level NO_2 exposures. Both usually occur simultaneously as a result of explosions and during artillery firing.

1.4. Mechanism of NO_2 action in biological systems

What are the morphological and pathophysiological manifestations of injury from exposure to very high levels of NO_2 (military exposure)? Is the mechanism of injury from very high level NO_2 similar to that of low level NO_2 , but only more intense? Several studies of the mechanism of action of NO_2 (Roehm et al., 1971; Menzel, 1976; Mustafa and Tierney, 1978; Pryor and Lightsey, 1981; Pryor et al., 1982; Elsayed and Mustafa, 1982; Selvanian et al., 1982; Sagai et al., 1982; Thomas et al., 1986) have suggested that NO_2, a free radical, would attack unsaturated fatty acids (RH) in the cell membrane, forming carbon centered radicals ($R^{\cdot}$) and oxygen centerd radicals ($ROO^{\cdot}$). These chain reactions can be broken and the free radicals quenched by antioxidants such as α-tocopherol (EH), to form non-propagating tocopheryl radicals ($E^{\cdot}$) according to the following reactions:

Reactions

$$NO_2 + RH \longrightarrow \longrightarrow \longrightarrow R^{\cdot} \qquad \text{(3) Initiation}$$

$$R^{\cdot} + O_2 \longrightarrow ROO^{\cdot} \qquad \text{(4) Peroxidation}$$

$$ROO^{\cdot} + RH \longrightarrow ROH + R^{\cdot} \qquad \text{(5a) Propagation}$$

$$ROO^{\cdot} + EH \longrightarrow ROH + E^{\cdot} \qquad \text{(5b) Antioxidation}$$

However, recent studies of the mechanism of O_3 toxicity suggested that another target for inhaled oxidants is more likely to be membrane proteins leading to their oxidation early on, thereafter lipid peroxidation takes place. Thus, the question now is would NO_2 react in a manner similar to O_3, a more powerful oxidant, but not a free radical? Should we change our long-held understanding of inhaled oxidants' mechanism of action regarding lipid peroxidation? If the early events reflect protein oxidation rather than lipid peroxidation, would our previous contention that lipid soluble antioxidants can help reduce NO_2 toxicity (Fig. 5) (Mustafa and Tierney, 1978; Sagai et al., 1982; Thomas et al., 1986; Roehm et al., 1971; Menzel, 1976; Pryor and Lightsey, 1981; Pryor et al., 1982; Elsayed and Mustafa, 1982; Selvanian et al., 1982; Fletcher and Tappel, 1973; Evans et al., 1981; Sagai and Ichinose, 1987; Leung and Morrow, 1981) still hold? Should we consider the use of water soluble antioxidants such as ascorbate (Selgrade et al., 1981; Mohsenin, 1987) to be an alternative to the lipid soluble antioxidants, or a

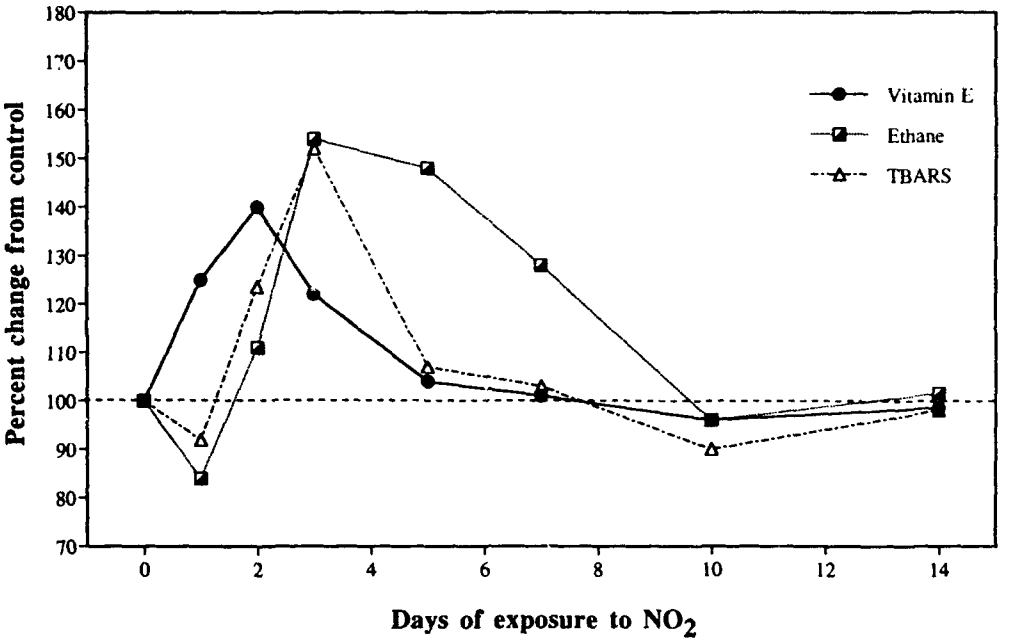

Fig. 5. Relationship between vitamin E and lipid peroxidation assessed by measuring ethane and thiobarbituric acid reactive substances (TBARS) production over time in lungs of rats exposed to 10 ppm NO_2. Modified from Sagai and Ichinose (1987).

combination of both (Mohsenin, 1991) or should we look for a new generation of antioxidants that has both hydrophilic and lipophilic antioxidant properties to be more effective in protecting both the cell membrane and cytosolic components? One such compound that may be promising is dihydrolipoic acid (Müller, 1989; Scholich et al., 1989; Busse et al., 1992).

Then there is an unanswered question of the time associated with antioxidant action. When does an antioxidant confer protection and for how long? Since alveolar type I cells are damaged by inhaled NO_2, they should also be the cells protected by antioxidants. In a study by Evans et al. (1981) this question was examined at the cellular level by feeding rats diets containing different levels of vitamin E, and selenium, a component of the antioxidant enzyme glutathione peroxidase, then quantitating the damage to type I cells after exposure to NO_2 for different periods. Results of that study indicated that antioxidants delayed the onset of damage in the early stages only between 6–12 h (Fig. 6A). However, as the exposure continued (24–48 h), there was no difference in protection (Fig. 6B) between the animals fed different dietary antioxidant levels. This finding may have military applications

since a majority of the exposures in military settings are for very short periods. Therefore, antioxidant supplementation of the soldier early on to raise their antioxidant levels before a potential exposure occurs, may afford protection when it is most needed. The use of aerosolized antioxidant is a method by which high concentration of antioxidants can be delivered quickly to the lung. In a preliminary study (Elsayed and Mead, unpublished data) administration of vitamin E aerosol to rats raised their lung tissue content 2–3-fold, and bronchoalveolar lavage by 300–400-fold immediately after ad-

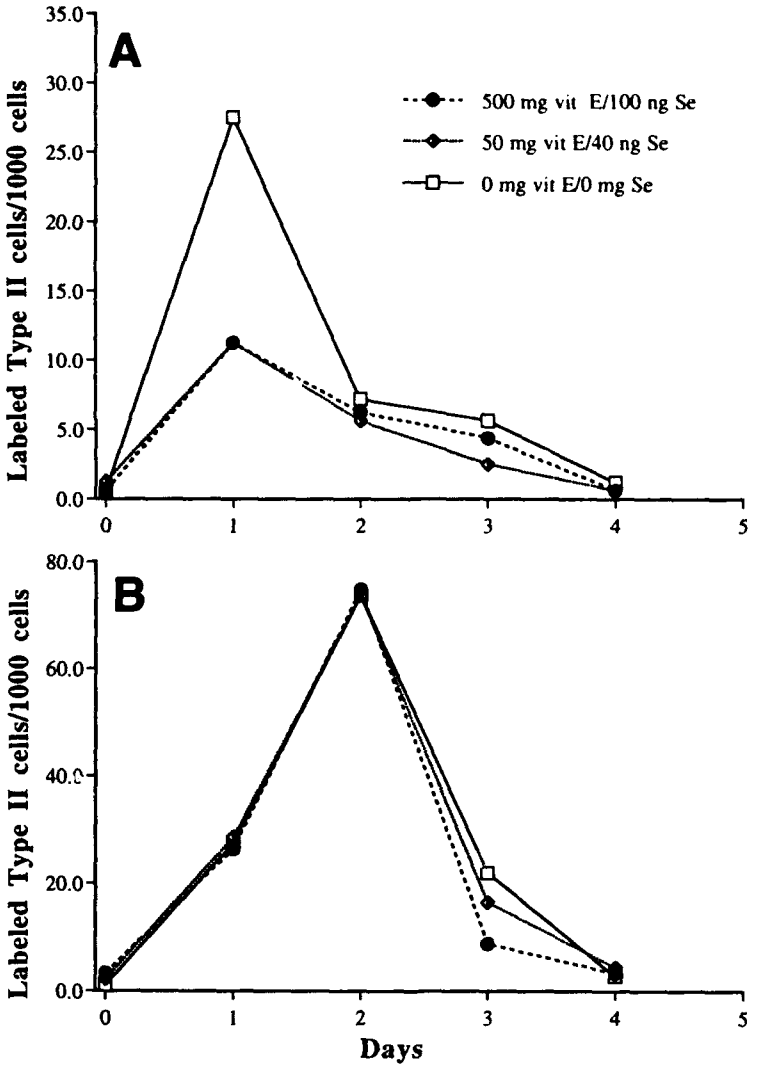

Fig. 6. Effect of dietary vitamin E and selenium supplementation on the proliferation of type II cells in the alveoli after exposure to 15 ppm NO_2. (A) exposure duration is 6 h. (B) exposure duration is 24 h. From Evans et al., 1981 by permission.

ministration. This treatment caused approximately 30% reduction in glucose 6-phosphate dehydrogenase activity in lung tissue after 24 h exposure to 1 ppm O_3.

1.5. Chemical interactions of NO_2

In an defeated (struck) tank or bunker, it is not realistic to assume that the exposure is limited to a single gas such as NO_2 which is strongly emphasized in military research. For example, it is more likely that in an atmosphere characterized by high heat and high concentrations of NO_2 and in the presence of environmental oxygen, O_3 would also be formed as shown in equations 1–3.

$$NO_2 \xrightarrow{\text{Heat or light } (\lambda < 430 \text{ nm})} NO^{\cdot} + O \quad (6)$$

$$O + O_2 \longrightarrow O_3 \quad (7)$$

$$NO^{\cdot} + O_3 \rightleftharpoons NO_2 + O_2 \quad (8)$$

Injury from exposure to a combination of NO_2 and O_3 is greater than the injury caused by each individual component due to the formation of new species more toxic than the original ones. Moreover, the effect can be more than the simple sum (additive) of the individual effect of each gas, i.e., it can be synergistic (Mustafa et al., 1984; Last and Warren, 1987; Ichinosa and Sagai, 1989; Lee and Mustafa, 1990; Last, 1991). This may result from exposure to the highly toxic N_2O_5 which would form then either break down or react further to form other toxic species as shown in reactions 9–13.

$$NO_2 + O_3 \longrightarrow NO_3 + O_2 \quad (9)$$

$$NO_3 + NO_2 \longrightarrow N_2O_5 \quad (10)$$

$$N_2O_5 \longrightarrow NO_2 + NO_3 \quad (11)$$

$$N_2O_5 + H_2O \longrightarrow 2HONO_2 \quad (12)$$

$$2HONO_2 \longrightarrow NO_3 + NO_2 + H_2O \quad (13)$$

In addition to the high intensity heat generated when a tank is struck by a High Energy Anti-Tank (HEAT) missile, despite the hard spent uranium shields, the metal body of the tank (or parts of it) will fragment. Some of the fragments may melt then vaporize exposing the crew to metal particulates including iron, aluminum and uranium oxide, to name a few, in addition to the

toxic gases (known and unknown) that have been mentioned. What is the toxicity of combined exposure to high level NO_2 and metal, carbon, or concrete particulates? The question has not been addressed yet, thus no answer is to be expected soon. Another very likely component of any complex exposure is the high energy impulse noise (blast overpressure) that usually accompanies penetration and explosion of in-coming missiles and/or explosion of on-board munitions. Understanding the underlying mechanism of injury by blast is now emerging. However, the effects of exposure to blast overpressure in combination with toxic gases and/or particulates may have a long time before being characterized.

Other possible NO_2 interactions in the defeated vehicles and tanks may occur when the seats and other polymeric fabric materials are burnt releasing halides and hydrocarbons. These compounds can interact with NO_2 either directly or indirectly in the presence of heat or metal catalysts as shown (reactions 14-18) to form new toxic compounds such as hydrochloric acid (HCl, reaction 15), carbon monoxide (CO, reaction 16), and hydrogen peroxide (H_2O_2, reaction 18).

$$Cl_2 \xrightarrow{(h\nu)} 2Cl \quad (14)$$

$$Cl + CH_2O \longrightarrow HCl + HCO \quad (15)$$

$$HCO + O_2 \xrightarrow{(+M)} HO_2 + CO \quad (16)$$

$$HO_2 + NO_2 \underset{}{\overset{(+M)}{\rightleftharpoons}} HO_2NO_2 \quad (17)$$

$$2HO_2 \longrightarrow H_2O_2 + O_2 \quad (18)$$

Studies of the effect of brief exposures to high concentrations of NO_2 using small and large animals were conducted by the Army as well as by civilian collaborating laboratories. Some of these studies will be presented in this symposium, but many more studies are still badly needed, particularly those on the effect of complex exposures to various realistic combinations of gases and particulates the soldier may have to face.

1.6. The symposium

The first paper in this symposium by Mayorga presents an overview of the military and civilian issues related to the effects of exposure to NO_2 particularly high level occupational and accidental exposures and the factors modifying the response. The second paper by Huie discusses the chemistry

of NO_2 in the gas phase and its rate constants, and examines the validity of several reactions proposed to occur in tissues. In the third paper Postlethwait, examines the kinetics of inhaled NO_2 absorption by the lung, using the isolated perfused rat lung, to elucidate the dosimetry and some reactions occurring on the alveolar air-liquid interface. Lehnert, then presents a comprehensive review of the work done in vivo using small animals (rats) to study the effects of brief exposure to high level NO_2. This will be followed by Januszkiewicz and Mayorga's account of in vivo studies using large animals (sheep). In their presentation, the relative toxicity of different routes of NO_2 exposure such as nose only exposures vs. intratracheal instillation will be compared. Finally, Mohsenin, discusses the human response to NO_2 exposure (low-levels) and the applicability of using the water soluble antioxidant, ascorbic acid in treatment, or to ameliorate that response.

2. References

Busse, E., Zimmer, G., Schopohl, B. and Kornhuber, B. (1992) Influence of α-lipoic acid on intracellular glutathione in vitro and in vivo. Arzneim.-Forsch./Drug Res. 42, 829.

Committee on Medical and Biological Effects of Environmental Pollutants. (1977) Nitrogen Oxides, National Academy of Sciences, Washington, D.C., p. 4.

Committee on Medical and Biological Effects of Environmental Pollutants. Ozone and other photochemical oxidants, National Academy of Sciences, Washington, D.C., 1977, p. 126.

Elasyed, N.M. (1993) Modulation of pulmonary vitamin E by environmental oxidants. In: L. Packer and J. Fuchs (Eds), Vitamin E in Health and Disease, Marcel Dekker, Inc., New York, p. 699.

Elsayed, N.M. and Mustafa, M.G. (1982) Dietary antioxidants and the biochemical response to oxidant inhalation. I. Influence of dietary vitamin E on the biochemical effects of nitrogen dioxide exposure in rat lung. Toxicol. Appl. Pharmacol. 66, 319.

Evans, M.J., Cabral, L.J.. Stephens, R.J. and Freeman, G. (1973) Renewal of alveolar epithelium in the rat following exposure to nitrogen dioxide. Am. J. Pathol. 77, 175.

Evans, M.J., Cabral-Anderson, L.J., Dekker, N.P., and Freeman, G. (1981) The effects of dietary antioxidants on NO_2-induced injury to type I alveolar cells. Chest 80, 5S.

Evans, M.J. (1982) Cell death and cell renewal in small airways and alveoli. In: H. Witschi and P. Nettesheim (Eds) Mechanisms in Respiratory Toxicology, CRC Press, Boca Raton, p. 189.

Fletcher, B.L., and Tappel, A.L. (1973) Protective effects of dietary tocopherol in rats exposed to toxic levels of ozone and nitrogen dioxide. Environ. Res. 6, 165.

Guidottio. T.L. (1980) Toxic inhalation of nitrogen dioxide: Morphologic and functional changes. Exp. Mol. Pathol. 33, 103.

Hayashi, Y., Kohno, T. and Ohwada. H. (1987) Morphological effects of nitrogen dioxide on rat lung. Environ. Health Perspect. 73, 135.

Ichinosa, T. and Sagai. M. (1989) Biochemical effects of combined gases of nitrogen dioxide and ozone. III. Synergistic effects on lipid peroxidation and antioxidative protective systems in the lungs of rats and guinea pigs. Toxicology 59, 259.

Lee, J.-S. and Mustafa. M.G. (1990) Effects of short-term, single and combined exposure to

low-level NO_2 and O_3 on lung tissue enzyme activities in rats. J. Toxicol. Environ. Health 29, 293.

Januszkiewicz, A.J., Snapper, J.R., Sturgis, J.W., Rayburn, D.B., Dodd, K.T., Phillips, Y.Y., Ripple, G.R., Sharpnack, D.D., Coulson, N.M. and Bley. J.A. (1992) Pathophysiologic responses of sheep to brief high-level nitrogen dioxide exposure. Inhal. Toxicol. 4,, 372.

Last, J.A. and Warren. D.L. (1987) Synergistic interaction between nitrogen dioxide and respirable aerosols of sulfuric acid or sodium chloride on rat lungs. Toxicol. Appl. Pharmacol. 90, 34.

Last. J.A. (1991) Global atmospheric change: potential; health effects of acid aerosol and oxidant gas mixtures. Environ. Health Perspect. 96, 151.

Leung, H.W. and Morrow. P.E. (1981) Interaction of glutathione and ascorbic acid in guinea pig lungs exposed to nitrogen dioxide. Res. Comm. Chem. Pathol. Pharmacol. 31, 111.

Menzel. D.B. (1976) The role of free radicals in the toxicity of air pollutants (nitrogen dioxide and ozone). In W.A. Pryor (Ed.), Free Radicals in Biology Vol. II, Academic Press, New York, p. 181.

Mohsenin. V. (1987) Effects of vitamin C on NO_2-induced airway hyperresponsivness in normal subjects. Am. Rev. Respir. Dis. 136, 1048.

Mohsenin. V. (1991) Lipid peroxidation and antielastase activity in the lung under oxidant stress: role of antioxidant defenses. J. Appl. Physiol. 70, 1456.

Muller. L. (1989) Protective effects of DL-α-lipoic acid on cadmium-induced deterioration of rat hepatocytes. Toxicology 58, 175.

Mustafa, M.G. and Lee. S.D. (1976) Pulmonary biochemical alterations resulting from ozone exposure. Ann. Occup. Hyg. 19, 17.

Mustafa, M.G. and Tierney. D.F. (1978) Biochemical and metabolic changes in the lung with oxygen, ozone, and nitrogen dioxide toxicity. Am. Rev. Respir. Dis. 118, 1061.

Mustafa, M.G., Elsayed, N.M., von Dohlen, F.M., Hassett, C.M., Postlethwait, E.M., Quinn, C.L., Graham, J.A. and Gardner. D.E. (1984) A Comparison of biochemical effects of nitrogen dioxide, ozone, and their combination in mouse lung. I Intermittent exposures. Toxicol. Appl. Pharmacol. 72, 82.

Mustafa, M.G., Faeder, E.J. and Lee. S.D. (1980) Biochemical effects of nitrogen dioxide on animal lungs. In: S.D. Lee (Ed), Nitrogen Oxides and their Effects on Health, Ann Arbor Science Publishers, Ann Arbor, MI, p. 161.

Ospital, J.J., Hacker, A.D. and Mustafa. M.G. (1981) Biochemical changes in rat lungs after exposure to nitrogen dioxide. J. Toxicol. Environ. Health 8, 47.

Pryor, W.A. and Lightsey, J.W. (1981) Mechanisms of nitrogen dioxide reactions: initiation of lipid peroxidation and the production of nitrous acid. Science 214, 435.

Roehm, J.N., Hadley, J.G. and Menzel. D.B. (1971) Antioxidants versus lung disease. Arch. Intern. Med. 128, 88.

Sagai, M. and Ichinose, T. (1987) Lipid peroxidation and antioxidative protection mechanism in rat lungs upon acute and chronic exposure to nitrogen dioxide. Environ. Health Perspect. 73, 179.

Sagai, M., Ichinose, T., Oda, H. and Kubota, K. (1982) Studies on biochemical effects of nitrogen dioxide. II. Changes of the protective systems in rat lungs and lipid peroxidation by acute exposure. J. Toxicol. Environ. Health 9, 153.

Samet J.M. and Utell. M.J. (1990) The risk of nitrogen dioxide: what have we learned from epidemiological and clinical studies? Toxicol. Industrial Health 6, 247.

Scholich, H., Murphy, M.E. and Sies. H. (1989) Antioxidant activity of dihydrolipoate against microsomal lipid peroxidation and its dependence on α-tocopherol. Biochem. Biophys. Acta 1001, 256.

Selgrade, M.K., Mole, M.L., Miller, F.J., Hatch, G.E., Gardner, D.E. and Hu, P.C. (1981) Effect of NO_2 inhalation and vitamin C deficiency on protein and lipid accumulation in the lung. Environ. Res. 26, 422.

Sevanian, A., Elsayed, N. and Hacker, A.D. (1982) Effects of vitamin E deficiency and nitrogen dioxide exposure on lung lipid peroxidation: use of lipid epoxides and malonaldehyde as measures of peroxidation. J. Toxicol. Environ. Health 10, 743.

Stavert, D.M. and Lehnert, B.E. (1990) Nitric oxide and nitrogen dioxide as inducers of acute pulmonary injury when inhaled at relatively high concentrations for brief periods. Inhal. Toxicol. 2, 53.

Stavert, D.M. and Lehnert. B.E. (1989) Potentiation of the expression of nitrogen dioxide-induced lung injury by postexposure exercise. Environ. Res. 48, 87.

Thomas, H.V., Muller, P.K. and Lyman. R.L. (1968) Lipoperoxidation of lung lipids in rats exposed to nitrogen dioxide. Science. 159, 532.

Yuen, T.G.H. and Sherwin. R.P., (1971) Hyperplasia of type II pneumocytes and nitrogen dioxide (10 ppm) exposure. Arch. Environ. Health 22, 178.